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Utilization of transgenic mice replicating high levels of hepatitis B virus for antiviral evaluation of lamivudine

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Abstract

A recently developed transgenic mouse strain which expresses high levels of hepatitis B virus (HBV) was studied as a model for evaluation of potential chemotherapeutic agents. Lamivudine ([−]2′-deoxy-3′-thiacytidine), known to reduce hepatitis B viremia in human patients, and zidovudine (3′-azido-3′-deoxythymidine), previously shown to be ineffective for HBV infections in man, were used in parallel in this transgenic animal model. Orally administered lamivudine at dosages of 100, 50, and 25 mg/kg per day given once a day for 21 days significantly decreased serum and liver HBV DNA titers in a dose-responsive manner. Zidovudine ($\sim$22 mg/kg per day) administered in the drinking water for 21 days was not effective in reducing these HBV parameters as compared to transgenic placebo-treated controls. The serum HBV DNA titers rebounded to high levels 1 week after cessation of lamivudine treatment. Male and female mice responded in a similar manner to these therapies. The results using this transgenic mouse model were similar to what would be predicted from treatment of HBV-infected human patients with lamivudine and zidovudine, and indicate these mice may be useful as a small animal chemotherapeutic model for study of potential HBV inhibitors.

Keywords: Lamivudine; Hepatitis B virus; Transgenic mice; Small animal chemotherapeutic model

1. Introduction

The generation of transgenic mice expressing hepatitis B virus (HBV) has been motivated in part by the expense and minimal availability of the HBV-chimpanzee model, and the absence of more convenient, small non-primate animal models that can be infected with HBV. The infections of ducks, woodchucks, and squirrels with their respective animal hepatitis viruses (Ganem, 1996) have been important in formulating much of our knowledge of HBV infection, but small animal

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models carrying the human virus have not been available previous to HBV-transgenic mice because of the limited host range of the virus.

The limitation of the host range of viruses can be partially overcome by genetically engineering animals to possess viral genomes such as has been demonstrated with human immunodeficiency virus (Leonard et al., 1988; Morrey et al., 1991), papilloma virus (Lacey et al., 1986), and the human hepatitis B virus (Chisari, 1995a,b; Guidotti et al., 1995; Chisari, 1996). Many transgenic mice have been produced that express portions of the HBV genome coding for the envelope (Farza et al., 1987; DeLoia et al., 1989; Gilles et al., 1992a,b; Ando et al., 1993; Koike et al., 1994), core (Guidotti et al., 1994c; Milich et al., 1994), precore (Milich et al., 1990; Guidotti et al., 1996), and X (Lee et al., 1990; Perfumo et al., 1992; Koike et al., 1994) proteins. The studies with these animals yielded good information on the viral induction, protein transport, assembly, secretion, disease pathology and HBV-specific immune responses. Such animals are generally not considered suitable for antiviral studies on viral replication, however, because they only express portions of the viral genome.

Initial attempts have been made to generate transgenic mice carrying the complete linear viral genome (Araki et al., 1989; Gilles et al., 1992a; Nagahata et al., 1992; Perfumo et al., 1992; Guidotti et al., 1994a,b; Tsui et al., 1995) with the anticipation that the effort would provide information on viral replication. These transgenic mice demonstrated that HBV can replicate in murine hepatocytes, but the virus was at low levels in the serum (Perfumo et al., 1992). Guidotti et al. (1995) have been successful in generating transgenic mice that replicate high levels of human hepatitis B virus in clinically important target organs of the liver and kidney. Pooled sera from these mice contain high titers of viral DNA approaching that found in the natural chronic human infection (Guidotti et al., 1995). Moreover, the profiles of HBV DNA replicative intermediate forms and RNA species in these transgenic mice were similar to those profiles identified in human and primate hosts. These are the transgenic mice used in this study.

The characteristics of the HBV infection in these transgenic animals suggest their potential utility as a model for study of anti-HBV drugs. To date, interleukin-12 has been studied in this mouse model (Cavanaugh et al., 1997); but further studies are also needed to establish if the model will yield antiviral data that are comparable to what has been reported in the human condition. This report describes the effects of two nucleoside analog inhibitors of reverse transcriptase on the HBV infection in these mice. These compounds are (−)2′-deoxy-3′-thiacytidine (lamivudine, 3TC) and 3′-azido-3′-deoxythymidine (zidovudine, AZT). Lamivudine has previously been shown to be effective for both treatment of HBV (Ling et al., 1996; Schnittman and Pierce, 1996) and HIV infections (Hart et al., 1992; Clumeck, 1993); zidovudine is highly efficacious for reducing HIV titers in human patients (Clumeck, 1993) and retrovirus titers in mice (Morrey et al., 1990; Ruprecht et al., 1990) and other animal species (Fazely et al., 1991; Tavares et al., 1997), but does not inhibit HBV infections in man (Mai et al., 1996). Thus, the effects of known positive and negative drugs in this model will aid in validating its potential for the study of anti-HBV drugs.

2. Materials and methods

2.1. Transgenic mice

Transgenic HBV mice obtained from Dr Francis V. Chisari (Scripps Research Institute, LaJolla, CA) were used. The animals were derived from founder 1.3.32 (Guidotti et al., 1995). Males and females were used to compare the suitability of both sexes for such chemotherapeutic experiments. The identity of each animal was tracked by toe clipping. Animal use and care was in compliance with the Utah State University Institutional Animal Care and Use Committee.

2.2. Serum HBV DNA assay

Since antiviral therapy was anticipated to significantly lower serum HBV DNA levels and since

limited quantities of blood were obtained from bleeding mice through the tail vein, PCR-based analysis was valuable to follow the progress of viremia. The PCR assay was semi-quantitative and was based on comparing intensities of sample PCR products with PCR products of HBV standards. A 360-bp region was chosen within the core gene sequence of the HBV genome to amplify using PCR. The oligonucleotide primers utilized for these analyses were complementary to the HBV sequence (accession # V01460) (Galibert et al., 1979). The forward primer sequence was GATTGAGACCTTCGTCTGCGAG (position 776–797) and the reverse primer sequence was CATTGTTCACCTCACCATACTGCAC (position 1146–1122).

Mouse serum samples were analyzed in duplicate. HBV DNA was prepared for amplification by extraction using GeneReleaser™ matrix (Bioventures, Murfreesboro, TN). For the extraction step, 5 μl of serum and 15 μl of GeneReleaser™ were added to 0.2-ml PCR reaction tubes and incubated in the thermocycler (Touchdown™, Hybaid, Middlesex, UK) using the following program: 37°C, 30 s/ 8°C, 30 s/ 65°C, 90 s/ 97°C, 180 s/ 8°C, 60 s/ 65°C, 180 s/ 97°C, 60 s/ 65°C, 60 s/ 80°C, 30 min. Thus, only one glove-change per serum sample was required for the lysis/DNA extraction step and the chance of loss due to pipetting errors during multiple organic extraction steps was eliminated. Following the lysis incubation procedure, the remaining components of the PCR reaction mixture were added (final volume of 40 μl) (Kaneko et al., 1989), and the reaction tubes were subjected to the following amplification program: 94°C, 2 min/ 94°C, 1 min/ 55°C, 1 min/ 72°C, 1 min/ (repeat steps 2–4, 39 times)/ hold at 4°C.

Following PCR amplification, 60 μl distilled, sterile, filtered water was added to each tube. Following 1 min of centrifugation ($15\,000 \times g$), the contents were applied to nitrocellulose membranes using a 96-well dot blot manifold (GIBCO-BRL, Gaithersburg, MD) and hybridized to a ^{32}P-labeled, 3.2-kb cloned HBV DNA fragment as previously described (Korba et al., 1986). HBV DNA content in the mouse sera was quantitated by comparison to a dilution series of chronic HBV carrier chimpanzee serum that contained a previously determined concentration of HBV DNA using an InstantImager™ beta scanner (Packard Instrument, Downers Grove, IL). This standard series, as well as appropriate negative control samples, was PCR amplified and dot blotted, in duplicate, each time the mouse sera were analyzed. The sensitivity of this HBV DNA detection procedure was approximately 500 HBV genome equiv./ml serum.

2.3. Liver HBV DNA assay

Hepatic HBV DNA was analyzed by Southern blot hybridization as previously described (Korba et al., 1989). Briefly, liver tissue samples were lysed in 4 M guanidine thiocyanate/7% 2-BME and centrifuged through a CsCl cushion. Whole cell DNA was then dialyzed, digested with proteinase K/SDS, extracted with phenol and chloroform, precipitated with ethanol, and suspended in 10 mM Tris–HCl (pH 7.5)/1 mM Na_2EDTA. DNA samples were digested with Hind III prior to electrophoresis. The hybridization probe was a ^{32}P-labeled full length cloned HBV genome. Samples were quantitated using a InstantImager beta scanner (Packard Instruments) against known amounts of cloned HBV DNA included in each gel.

2.4. Serum hepatitis antigen assays

Abbott HBeAg (rDNA) and HBsAg (Auszyme™ Monoclonal) assays were used to identify HBV antigens in the serum of transgenic mice. The assays were performed according to the manufacturer's instructions. Standard curves were constructed using purified HBeAg (Biodesign International, Kennebunk, ME) and recombinant HBsAg (Fitzgerald Industries International, Concord, MA). The antigen concentrations of the samples at a 1/30 dilution were then determined from the standard curves.

2.5. Drugs

Lamivudine was obtained from Dr Bud Tennant (Cornell University, Ithaca, NY). It was

prepared in sterile physiological saline and stored at 4°C until used. Zidovudine, obtained from GlaxoWellcome (Research Triangle Park, NC), was prepared in sterile double-distilled water at a dose of 0.2 mg/ml and stored at 4°C until used. It was administered ad libitum via the drinking water, which was changed daily. Based on the quantity of water consumed by mice, this dosage of zidovudine was approximately 22 mg/kg per day and has previously been shown to strongly inhibit retroviral infection in mice (Morrey et al., 1990).

2.6. Experimental design

Serum from all mice was initially assayed for HBsAg and HBeAg. HBsAg values of 0.04 optical density (O.D.) or higher, and HBeAg values of 0.02 O.D. or higher were considered to be indicative of positive HBV transgenic mice which were then used in this chemotherapy experiment. The male or female HBV transgenic animals were treated per os (p.o.) twice a day for 21 days with 100, 50, or 25 mg/kg per day of lamivudine. Eight to twenty-four mice (both sexes) were included in each treatment group, depending on the assay day and treatment group. High- and low HBsAg- and HBe-Ag-expressing mice were assigned to each group. Blood was collected from the tails on days 0, 7, 14, 21, 28, and 35. Three male and three female mice from each group were randomly euthanized for liver removal on day 22, which was the day after the last drug treatment. Livers were snap-frozen in liquid nitrogen until processed for hybridization (Korba et al., 1989) for detection of HBV DNA intermediates. As a control, an equal number of saline-treated transgenic mice were run in parallel. As a negative control, a group of transgenic mice were treated with zidovudine for 21 days. The viral parameters of individual mice were recorded so that the effect of drug could be monitored over time for each individual animal.

2.7. Statistical analysis

Differences between mean values were analyzed using the t-test. Standard deviations were also determined. χ^2 analysis with Yates' correction was used with numbers of serum samples yielding no detectable levels of HBV DNA, or with the number of HBsAg or HBeAg titers above 200 ng/ml of compared to the total number of samples.

2.8. Personnel safety measures

The animal work was carried out in the Biosafety Level 3 (BL-3) suite of the Utah State University Laboratory Animal Research Center. All personnel who entered the BSL-3 area were required to receive the current commercially available hepatitis B vaccine as administered by the Utah State Health Department. All personnel received special training on blood-borne pathogen handling by this university's Environmental Health and Safety Office.

3. Results

3.1. Viral parameters

The means $\pm$ S.D. of the serum HBV DNA, and the numbers of samples with titers above 200 ng/ml of serum HBeAg or HBsAg were calculated for male and female animals to determine if the females were different than males possibly due to factors such as hormonal fluctuations (Farza et al., 1987). The mean and variability of the serum HBV DNA titers were essentially the same between male and female mice (Table 1). The percentage of HBsAg values above 200 mg/ml for the male mice was essentially the same as those for the female mice. The percentage of serum HBeAg

Table 1
A comparison of serum HBV DNA and serum viral antigen titers between male and female transgenic mice

	Males	Females
HBV DNA[a]	5.4 ± 1.0	5.2 ± 0.7
HBsAg[b]	21/65 (32.3%)	22/51 (43.1%)
HBeAg[b]	33/65 (50.8%)	2/51* (3.9%)

[a] Mean $\log_{10}$ HBV DNA (genome equiv./ml) $\pm$ S.D.
[b] Number of mice with serum antigen titers above 200 ng/ml over total; percentage in parentheses.
* $P<0.001$ as compared with male values.

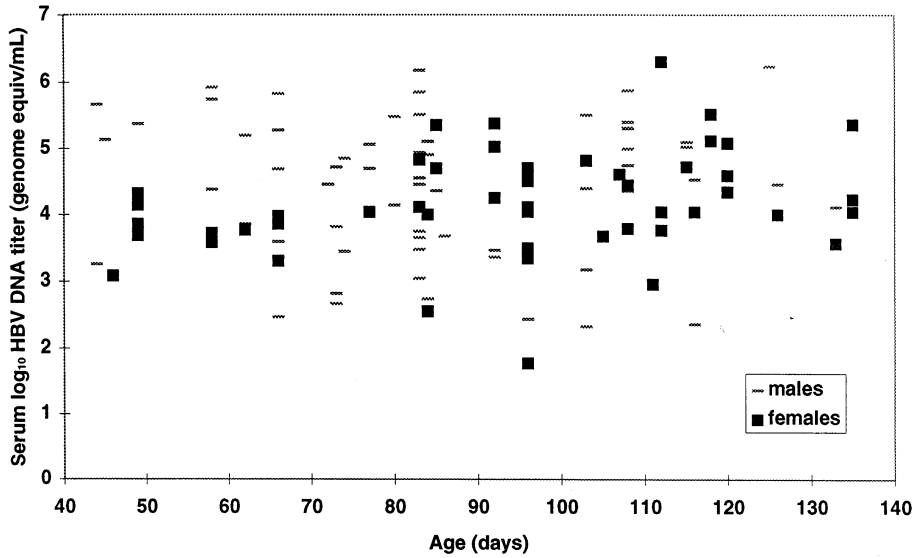

Fig. 1. Correlation of serum HBV DNA with age of transgenic mice not receiving drug treatments.

titers above 200 ng/ml in the females (2/51, 3.9%), however, was lower ($P < 0.01$) than the values for male animals (33/65, 50.8%). Experiments involving HBeAg parameter should take into account this observation of differential expression of HBeAg in female mice as compared to male mice. These data indicate, however, that females are as suitable for HBV chemotherapeutic studies as males in terms of serum HBV DNA and HBsAg.

There was a possibility that some variability of serum HBV DNA titers might be attributable to age of the animals, since it has been previously observed that viral parameters increase from near the limits of detection at birth to full expression by 4 weeks old (Guidotti et al., 1995). The serum HBV DNA titers of animals with a wide range of ages (44–135 days) were compared. The data indicated that there was no correlation between ages of mice in either sex and the sera HBV DNA titers (Fig. 1).

A possible approach for reducing variability would be to group mice that possess similar serum antigen titers with the expectation that these animals would have similar high serum HBV DNA titers. To analyze this approach, the two serum antigens, HBsAg and HBeAg, were plotted against the serum HBV DNA titers of individual mice to determine if there was a significant correlation (Fig. 2). Neither HBsAg (A panel) or HBeAg (B panel) titers correlated with serum HBV DNA titers with either sex.

3.2. Drug treatments

A comparison of the values of the drug-treated mice with the values of saline-treated control mice or with values at the beginning of the experiment before treatment was initiated (day 0) is shown in Table 2. All dosages of lamivodine at days 14 and 21 post-initial treatment significantly ($P < 0.01$) reduced virus load (serum HBV DNA) when compared to the values from the same animals assayed before initiation of treatment. Moreover, this reduced viral titer was also statistically significant ($P < 0.01$) when compared to saline-treated control mice at the same days of treatment. Values collected at day 7 from mice treated with 100 or 50 mg/kg per day of lamivudine were also significantly ($P < 0.01$) reduced when compared to the values from the same animals at day 0.

Dose-response effects of lamivudine can be identified from a graphic overview represented in Fig. 3. By comparing the serum HBV DNA titers of different dosages on days 14 or 21 after initia-

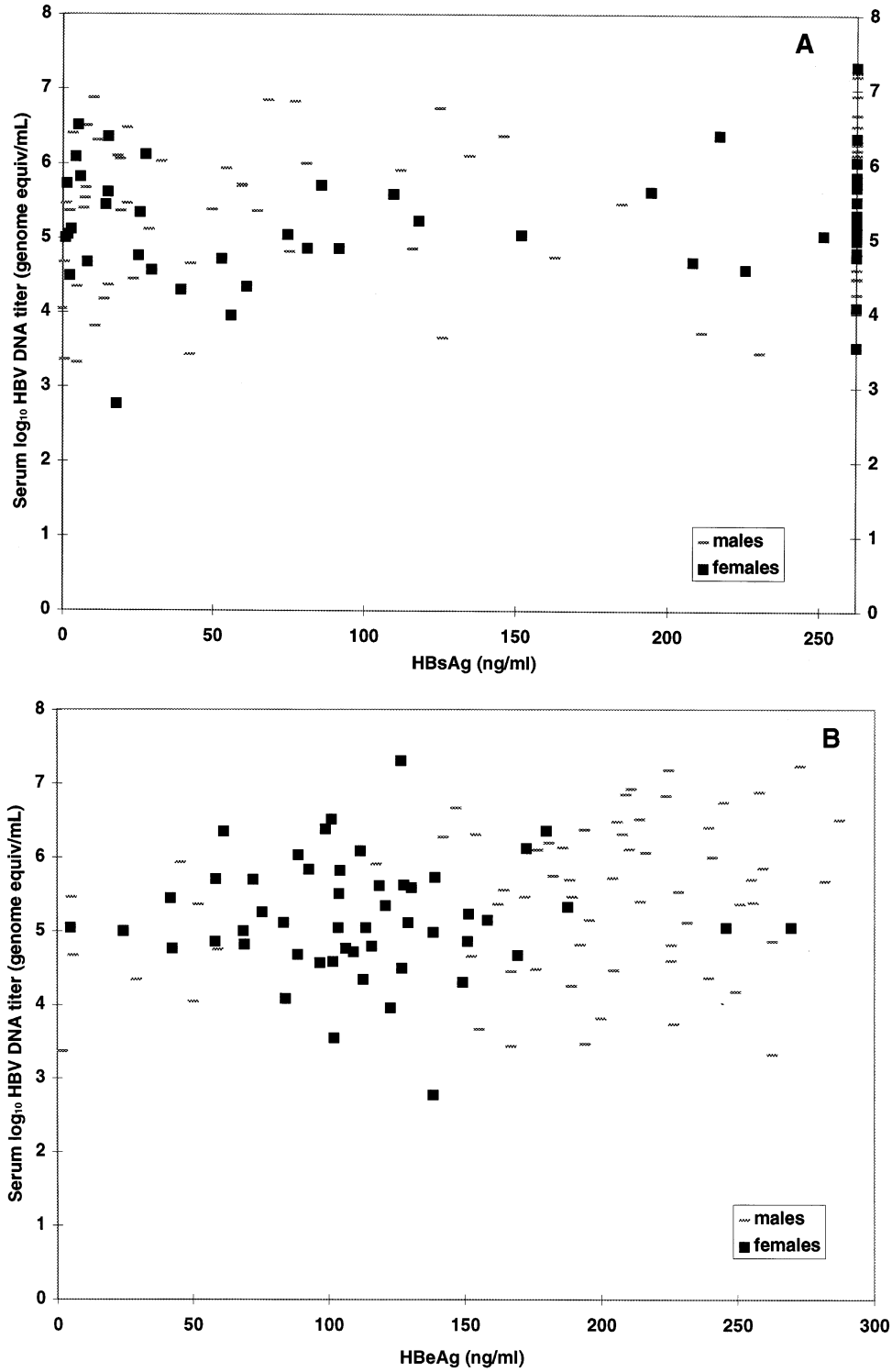

Fig. 2. Correlation of serum HBV DNA with serum HBsAg (A) and HBeAg (B) titers in transgenic mice not receiving any drug treatments.

tion of lamivudine oral treatment, a dose-responsive decline in titers can be identified with increasing concentrations of drug (25–100 mg/kg per day). A reduction in titers occurred as the treatments progressed from days 7 through 21 in mice treated with any dosage. The titers for each individual mouse were recorded so that the effect of drugs could be monitored over time for each animal. All mice except for mouse #272 (15/16, 94%) did not have detectable levels of serum HBV DNA after the last day of high-dosage lamivudine treatment (100 mg/kg per day). HBV DNA titers were monitored after cessation of lamivudine treatment on day 21. By 7 days after treatment terminated, the serum HBV DNA titers had at least the original titers found in mice on day 0.

Zidovudine was administered in parallel with the lamivudine at a concentration shown to be highly efficacious in Friend virus-infected mice (Morrey et al., 1990). This treatment did not affect the serum HBV DNA titers in the transgenic mice (Table 2) when the titers were statistically compared with the values of saline-treated control mice at the same days of treatment. In the saline-treated animals, titers at day 21 were significantly lower ($P \leq 0.01$) than the titers at the beginning of the experiment at day 0 (Table 2). A similar reduction was also observed in titers of zidovudine-treated animals; however, the titers from the zidovudine-treated mice were not significantly different from the saline-treated mice at any time point during the course of the experiment.

HBV DNA replicative intermediates (RI) were also assayed 1 day after the last treatment (day 22) in the livers of transgenic mice (Table 3). Oral treatment with the highest dosage of lamivudine (100 mg/kg per day) significantly ($P < 0.01$) reduced HBV DNA RI when compared to saline-treated control mice.

4. Discussion

Sustaining a strong, effective reduction in virus load in HBV transgenic mice may result in reduced liver disease and the development of hepatocellular carcinoma. Also, a reduction in this

Table 2
Effect of orally administered lamivudine and zidovudine[a] on serum HBV DNA in HBV-transgenic mice (both sexes)

Compound	Dose (mg/kg per day)	Serum $\log_{10}$ HBV DNA titer[b] on day[c]					
		0	7	14	21	28	35
Lamivudine	100	5.6 ± 0.8 (24)	$3.4 \pm 1.1^{\dagger\dagger}$ (18)	$2.8 \pm 1.0^{**,\dagger\dagger}$ (17)	$2.5 \pm 0.5^{**,\dagger\dagger}$ (16)	5.6 ± 2.2 (8)	$4.2 \pm 1.1^{\dagger\dagger}$ (8)
	50	5.5 ± 1.5 (24)	$3.8 \pm 1.5^{*,\dagger\dagger}$ (18)	$2.8 \pm 0.9^{**,\dagger\dagger}$ (16)	$2.6 \pm 0.5^{**,\dagger\dagger}$ (16)	$7.3 \pm 0.9^{**,\dagger\dagger}$ (10)	5.8 ± 0.8 (10)
	25	5.2 ± 0.9 (23)	4.7 ± 1.4 (17)	$3.4 \pm 1.1^{**,\dagger\dagger}$ (17)	$3.1 \pm 1.1^{**,\dagger\dagger}$ (16)	5.0 ± 1.2 (10)	4.6 ± 1.3 (10)
Zidovudine	22	4.9 ± 1.1 (21)	4.7 ± 1.1 (18)	4.2 ± 1.5 (15)	$4.0 \pm 1.3^{\dagger}$ (15)	5.0 ± 1.3 (12)	4.2 ± 1.1 (9)
Saline	–	5.5 ± 1.1 (24)	5.1 ± 1.3 (24)	5.0 ± 0.9 (24)	$4.4 \pm 1.4^{\dagger\dagger}$ (24)	5.3 ± 1.2 (18)	5.0 ± 1.4 (18)

[a] HBV-transgenic mice (founder 1.3.32) were orally treated twice daily with lamivudine or saline for 21 days. Zidovudine was administered in the drinking water for 21 days at a concentration of 0.2 mg/ml. Mice were bled from the tails to obtain serum.
[b] Expressed as genome equiv./ml $\pm$ S.D.; no. of animals in parentheses.
[c] Days post-initial treatment.
* $P < 0.05$, compared to saline-treated controls at the same sampling day.
** $P < 0.01$, compared to saline-treated controls at the same sampling day.
† $P < 0.05$, compared to day 0 value in the same group.
†† $P < 0.01$, compared to day 0 value in the same group.

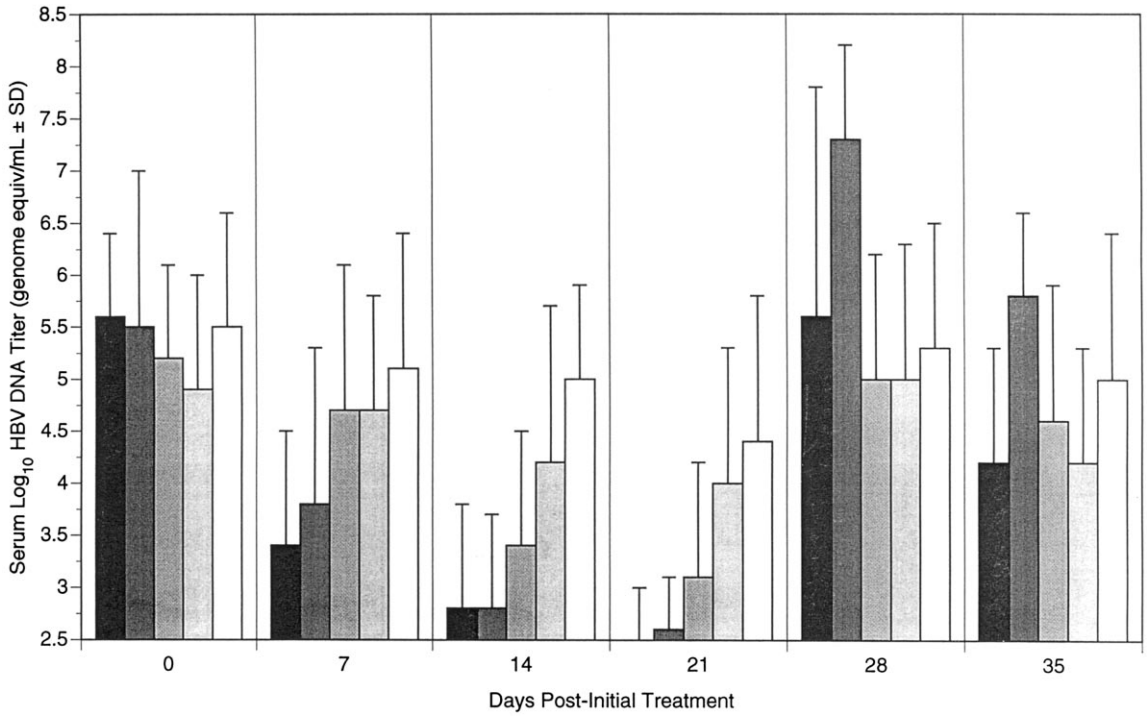

Fig. 3. Effect of orally administered lamivudine (3TC) or zidovudine (AZT) on serum HBV DNA in transgenic mice during and after last treatment at 21 days. 3TC was administered i.p., bid for 21 days; AZT was administered in the drinking water for 21 days. Serum was assayed for HBV DNA by quantitative PCR every 7 days until day 35, 2 weeks after the last treatment. Solid pattern, 3TC, 100 mg/kg per day; 75% fill pattern, 3TC, 50 mg/kg per day; 50% fill pattern, 3TC, 25 mg/kg per day; 25% fill pattern, AZT, ~22 mg/kg per day; no pattern, Saline.

parameter may prevent the emergence of mutant drug-resistant viruses (Moriya et al., 1996; Marcellin et al., 1997). Therefore, a small animal model which will demonstrate virus replication and load is important for developing therapies. The results of this study suggest that the HBV transgenic mice employed in this experiment may be useful as this animal model.

Part of the life cycle of HBV involves a viral DNA polymerase to reverse transcribe RNA to ssDNA and to transcribe ssDNA to partial ds-DNA. Consequently, a potential chemotherapeutic target for HBV is the viral polymerase using nucleoside analogues. Nagahata et al. (1992) have reported a reduction of liver HBV DNA in a small number of transgenic mice using treatment with a nucleoside analogue, oxetanocin G. The transgenic mice in that study, however, did not produce detectable full-length viral RNA or high levels of serum HBV DNA as indicators of infectious viral particles. The transgenic mice of the present study do produce both of these important parameters (Guidotti et al., 1995). Oral lamivudine at all dosages significantly reduced serum HBV DNA in a dose-responsive manner. The HBV liver DNA correlated with this reduction in serum HBV DNA which suggested that 3TC treatment had a true antiviral effect rather than a secondary effect of reducing the serum HBV DNA and not the liver HBV DNA. Zidovudine treatment was not effective in reducing serum HBV DNA titers. These results are the predicted outcome based on human studies using both drugs (Hart et al., 1992; Ling et al., 1996; Mai et al., 1996; Schnittman and Pierce, 1996).

Cessation of lamivudine treatment in clinical HBV studies resulted in a subsequent increase in viral parameters (Marinos et al., 1996). Because of

this observation in human subjects and our attempts to validate the usefulness of this model, the HBV DNA titers were monitored after cessation of treatment. A profound increase in titers occurred 1 week (day 28) after the last treatment in mice treated with all dosages of lamivudine, which further helped to validate the usefulness of the model.

Despite the usefulness of this PCR assay in this study, its use also had some disadvantages in that it was only semi-quantitative and the assay was possibly subjected to contaminating HBV DNA from the transgene through lysed cells during the serum collection. Nevertheless, the HBV PCR assay identified drug dosage- and time-responsive titers (Table 2, Fig. 3), which indicated its usefulness. The use of the semi-quantitative PCR assay probably contributed to variability of serum HBV DNA values. Such variability might be reduced by using an internal standard in the PCR reaction that is identifiably different from the sample HBV PCR product (Erhardt et al., 1996). Improvement to this PCR assay might increase the utility of this HBV transgenic mouse model for future studies. Moreover, if more blood were collected from each individual mouse, perhaps by orbital eye bleeding (Riley, 1960), assays not using PCR technology, such as dot blot analysis, would circumvent inherent problems with PCR, such as contamination with the HBV transgene in lysed serum samples. Titers might be too low, however, for detection of serum HBV in drug-treated mice despite the increased volume of serum that might be obtained. Despite its deficiencies, the current PCR assay was sufficient to observe profound differences in serum HBV DNA titers between treatment groups. Also, the low HBV DNA titers in the serum of mice treated with the highest concentration of lamivudine correlated with low liver HBV DNA titers.

HBV surface antigens in transgenic mice have been found to be regulated by sex steroids and glucocorticoids (Farza et al., 1987). This raised the possibility that the female murine values might vary due to hormone fluctuations. The means and variability of serum HBV DNA and HBsAg were essentially the same between male and female mice, indicating that females are as suitable for HBV chemotherapeutic studies as male mice in terms of variability. The usability of females is fortuitous because they do not need to be housed in separate cages as do the males. The biological significance of female mice possessing significantly lower serum HBeAg titers, as compared to male mice, is not known, but this observation does not preclude the use of female mice in such chemotherapeutic studies.

Two other approaches, besides monitoring variability between sexes, were tested to reduce animal-to-animal variability of serum HBV DNA titers. These approaches were to correlate the HBV DNA with either age or with serum antigen titers. If strong correlations were to exist between these parameters, transgenic mice could be

Table 3
Effect of orally administered lamivudine and zidovudine[a] on liver HBV DNA in transgenic mice

Compound	Dose (mg/kg per day)	Liver HBV DNA (day 21 post-treatment; mean pg/μg cell DNA ± S.D.) ($n^b = 6$)
Lamivudine	100	0.9 ± 0.7**
	50	2.5 ± 2.2
	25	7.9 ± 11.4
Zidovudine	22	2.5 ± 2.2
Saline	–	5.5 ± 5.7

[a] HBV-transgenic mice (founder 1.3.32) were orally treated twice daily with lamivudine or saline for 21 days. Zidovudine was administered in the drinking water for 21 days at a concentration of 0.2 mg/ml. Three male and three female mice were sacrificed in each treatment group to process livers for HBV replicative DNA intermediates.

[b] Number of animals in each treatment group.

** $P < 0.01$, compared to saline-treated controls at the same sampling day.

screened and selected for use in chemotherapeutic experiments to reduce variability. Nevertheless, results indicated that there was no correlation of serum HBV DNA with either age or serum antigen. A simple explanation for a lack of correlation of these parameters might be the inadequacies of the semi-quantitative PCR. Refinement of the PCR assay might yield better correlation of serum HBV DNA with age or serum antigens for future studies. Despite the potential problems of the semi-quantitative PCR assay, statistically significant efficacy was readily identified with the treatment of lamivudine if sufficient numbers of animals were used and if the parameters were monitored within each individual mouse.

A gradual reduction in serum HBV DNA titers was observed in saline-treated animals up to day 21 during the course of oral gavage treatment. After cessation of oral treatment, the titers of saline-control mice were restored to pre-treatment levels. A possible cause for the decline in titers might be sequential handling and blood letting from the tail. The animals were oral gavaged twice per day for 21 days and a significant quantity of blood was taken once every week during the duration of the experiment. It is possible the replenishment or dilution of blood in animals might have outdistanced the production of viremia resulting in a slight diminution of titer. Certainly considerable stress was involved in the continuous treatments which might have played a role in this observation. Once the treatment was stopped, however, the titers of the control animals increased slightly which may have resulted from a reduction of stress or oral gavage treatment.

This HBV-transgenic mouse model does have some disadvantages. Some of these are: (1) no HBV-induced pathology or death is observed with these animals (Guidotti et al., 1995); (2) episomal supercoiled DNA intermediate (cccDNA) is not detectable and the replicating genome is mostly driven from the transgene (Guidotti et al., 1995), and (3) infection of uninfected cells cannot be evaluated since HBV is not infectious for murine cells and all cells in the transgenic mouse contain the HBV transgene.

Some advantages of this model are that (1) this study demonstrated that the model is predictive for two nucleoside analogues; (2) the transgenic mouse model uses the human virus (Ganem, 1996); (3) mouse maintenance costs are relatively low; (4) less drug is required for treatment of mice as compared with larger animals; (5) large numbers of the mice can be used for statistical power, and (6) inbred mice have defined genetics for immunological and virological experimentation. These advantages indicate this to be a valuable therapeutic model for HBV.

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